GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic composition of a Brazilian population: the footprint of the Gold Cycle

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Genet. Mol. Res. 12 (4): 5124-5133 (2013)
Received March 28, 2013
Accepted September 20, 2013
Published October 28, 2013
DOI http://dx.doi.org/10.4238/2013.October.29.6

ABSTRACT. Ancestry-informative markers (AIMs) are powerful tools for inferring the genetic composition of admixed populations. In this study, we determined the genetic ancestry of the Ouro Preto (Brazil) population and evaluated the association between ancestry and self-reported skin color. The genetic ancestry of 189 children

and adolescents was estimated by genotyping 15 AIMs. The estimate of population admixture was determined using the Bayesian Markov Chain Monte Carlo (MCMC) method implemented in two different programs (STRUCTURE and ADMIXMAP). Volunteers self-reported their skin colors. The European ancestry contribution ranged from 0.503 to 0.539, the African contribution ranged from 0.333 to 0.425, and the Amerindian component ranged from 0.04 to 0.164. The relative contributions of African ($P < 0.016$) and European ($P < 0.011$) ancestry differed significantly among skin color groups, except between black and dark-brown groups. The population of Ouro Preto has a higher contribution of African ancestry compared to the mean for the southeast region of Brazil. Therefore, extrapolating the African ancestry contribution for southeastern Brazil to the Ouro Preto population would underestimate the actual value for this city. We also showed that self-reported skin color could be appropriate for describing the genetic structure of this particular population.

Key words: Ancestry-informative markers; Genetic ancestry; Skin color; Brazilian population

INTRODUCTION

Studies using ancestry-informative markers (AIMs) have been conducted in admixed populations for several applications including anthropological studies, forensic investigations, correlation studies of race, ethnicity, and ancestry, biomedical research, and pharmacogenetics (Via et al., 2009). For instance, population stratification, a potential confounding factor that can lead to false-positive or false-negative results (Thomas and Witte, 2002), can be detected and corrected for using specific statistical methods (Tiwari et al., 2008) if ancestry is known.

Studies using AIMs raised the question as to whether a social classification system based on race using self-reported skin color is appropriate in admixed populations. For instance, Instituto Brasileiro de Geografia e Estatística (IBGE) uses the color classification system in their household surveys and considers five skin color groups (white, black, yellow, brown, and indigenous) (IBGE, 2011). However, the validity of this color classification system is questionable for admixed populations because it is based on a subjective trait and apparently does not distinguish individuals by ancestral groups (Parra et al., 2003; Pimenta et al., 2006; Suarez-Kurtz et al., 2007; Santos et al., 2009; Lins et al., 2011; Pena et al., 2011; Leite et al., 2011). Moreover, self-identification has been shown to change over time (Hitlin et al., 2006; Santos et al., 2009), and even between sibling pairs, there was no satisfactory agreement between self-reported skin color groups or between self-reported skin color and melanin levels (Leite et al., 2011). According to Pena (2007), morphological traits are poor indicators of ancestral origin; therefore, this type of classification is merely social and may lead to misinterpretations in epidemiological studies.

The Brazilian population is formed by the mixture of three ancestral groups: European, African, and Amerindian (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011). The European contribution is the most significant (70.7-77.1%), followed by African (14.3-

16.9%) and Amerindian (8.5-11%) contributions (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011). However, these studies have been mainly conducted with individuals from the large urban centers of the five Brazilian geopolitical regions (North, South, Northeast, Southeast, and Midwest), and the peculiarities of small towns and cities associated with their geographic isolation, the influence of other regions according to their proximity, and/or the colonization process have been relatively neglected. For instance, the city of Montes Claros in the state of Minas Gerais has an African contribution of 39% (Silva et al., 2010), which is higher than the overall African contribution in southeast Brazil (14.1-27%) (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011; Giolo et al., 2012). In this case, the geopolitical proximity to the state of Bahia, located in the northeast region with an African ancestry proportion of 29.3% (Pena et al., 2011), may have influenced the population structure.

Data from the 2010 population census (IBGE) showed that the frequencies of individuals who self-reported their skin color as black (14.4%), brown (52.5%), yellow (1.6%), or indigenous (0.3%) in Ouro Preto (a small town in the state of Minas Gerais, southeast Brazil) were higher than the mean frequencies described for the state of Minas Gerais. These differences (5.2% higher for black skin and 8.2% higher for brown skin) reflect the structure of this population, which was formed at the time of the Gold Cycle by a mixture of Europeans, citizens from São Paulo and northeastern Brazil, African slaves, and Amerindians (Werkema, 2010). Recently, a high prevalence of risk factors and high morbidity (27.5%) due to cardiovascular diseases has been observed in this population (IBGE, 2010). Thus, as the prevalence of complex diseases such as cardiovascular diseases may vary among ethnic groups (Cossrow and Falkner, 2004; Wang and Beydoun, 2007; Vieira et al., 2009; Sekhobo et al., 2010), studying the genetic composition of this population can facilitate interpreting the results of previous and future epidemiological studies on this population.

In the present study, we describe the genetic structure of the population of Ouro Preto, Minas Gerais, Brazil using 15 AIMs and evaluate the association between genetic background and self-reported skin color.

MATERIAL AND METHODS

Study population

The study sample consisted of 189 volunteers (10.6 ± 2.04 years of age) randomly selected from a cross-sectional population-based study conducted in 2006 in the city of Ouro Preto, Brazil (Cândido et al., 2009). The volunteers donated a blood sample that was used for the extraction of genomic DNA (as determined by Miller et al., 1988) and answered a multiple-choice questionnaire about their skin color, which was self-reported as white, light brown, dark brown, black, or undeclared. As all participants were underage, their legal guardians signed a consent form, and the project was approved by the Research Ethics Committee of the Federal University of Ouro Preto.

Ancestral populations

Three groups of individuals (Africans, Europeans, and Amerindians) were used as reference populations for this study. The African sample (AFR, N = 120) was composed of

individuals from Senegal, Ghana, Cameroon, and Botswana. The European sample (EUR, N = 78) was composed of individuals from Chicago and Baltimore, USA, and the Amerindian sample (AMR, N = 29) was composed of individuals of the Zapotec people from Mexico. The genotypes of the reference populations were provided by Smith et al. (2004).

Selected markers

Fifteen AIMs were selected to evaluate the genetic mixture of volunteers: rs1426654, rs2065160, rs730570, rs1129038, rs1240709, rs3796384, rs2278354, rs2814778, rs1480642, rs6034866, rs7349, rs803733, rs222541, rs267071, and rs730086. The markers are distributed among chromosomes 1, 3, 5, 6, 9, 10, 14, and 15, and were previously tested by Lins et al. (2010). These markers produced ancestry estimates for the Brazilian population that were consistent with the expected heterogeneity. These genetic markers were chosen because they show more than 40% differences in allele frequencies between the reference populations (European and African) (Lins et al., 2010).

Genotyping assay

The gene fragments were co-amplified in a 5 µL volume that contained 30 ng genomic DNA, 0.2 µM primers, and 1X Qiagen Multiplex PCR Master Mix (Qiagen). The multiplex polymerase chain reaction (PCR) conditions were 95°C for 15 min, followed by 39 cycles of 30 s at 94°C, 90 s at 57°C, and 60 s at 72ºC, and a final extension of 10 min at 72°C. After the amplification, 3 µL PCR product was subjected to enzymatic digestion using 1 U/µL *Escherichia coli* exonuclease I (USB), 0.5 U/µL shrimp alkaline phosphatase (USB), and 0.5X shrimp alkaline phosphatase buffer. The reaction was incubated at 37°C for 60 min, followed by incubation at 75°C for 15 min. This treatment removes unincorporated primers and nucleotides.

The identification of the alleles for each marker was performed by a single-base extension (SBE) multiplex reaction using the ABI Prism® SNaPshot™ Multiplex kit (Applied Biosystems). The reaction was performed in a 5 µL volume that contained 2 µL purified PCR product, 1.66 µL SNaPshot™ Kit Reaction Mix, and 0.55 µM primers, except for rs1480642 (0.8 µM), rs1240709 (0.4 µM), and rs7349 (0.4 µM). The PCR conditions were 96°C for 2 min, followed by 30 cycles of 10 s at 96°C, 10 s at 58°C, and 20 s at 60°C. After the SBE multiplex reaction, a new enzymatic digestion was performed with 0.5 U/µL shrimp alkaline phosphatase (USB) and 0.5X shrimp alkaline phosphatase buffer.

For polychromatic electrophoresis, a mixture containing 1 µL purified product of the SBE multiplex reaction, 8.8 µL Hi-Di formamide, and 0.2 µL Liz120 internal standard (Applied Biosystems) was analyzed using an ABI Prism Genetic Analyzer 3100 sequencer (Applied Biosystems).

The sequences of all primers used in this study are available in the supplementary material of Lins et al. (2010).

Statistical analysis

The estimate of population admixture was calculated using the Bayesian Markov Chain Monte Carlo (MCMC) method, which was implemented using STRUCTURE ver-

sion 2.3.3 software (Pritchard et al., 2000; Falush et al., 2003) and the ADMIXMAP (http://homepages.ed.ac.uk/pmckeigu/admixmap/) program. The genotypes of the Ouro Preto sample population and the reference populations were analyzed together in STRUCTURE, assuming K = 3. The following parameters were used in STRUCTURE: an admixture and correlated allele frequency model, a burn-in period of 80,000, and 50,000 extra MCMC repetitions. In ADMIXMAP, we used 2500 iterations for the burn-in period and 10,000 iterations to measure the parameter data. The allele frequencies were determined by gene counting using individual genotyping data and were tested for Hardy-Weinberg equilibrium. One-way analysis of variance (ANOVA) followed by the Games-Howell post-hoc test was used to test for differences in the proportions of each ancestry type between the different self-reported skin color groups. The statistical analyses were performed using the SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). The significance level was set at $P < 0.05$.

RESULTS

Of the 189 individuals selected for this study, 101 were female (53.4%) and 88 were male (46.6%). Eleven individuals (5.8%) did not report skin color and were excluded from the analyses. The allele frequencies for the 15 AIMs in the ancestral populations reported by Lins et al. (2010) and in this study population (overall, and according to the self-reported skin color) are shown in Table 1. The allele frequency distributions of all single nucleotide polymorphisms (SNPs) were in Hardy-Weinberg equilibrium, except for markers rs1426654 and rs803733.

Table 1. Allelic frequencies and information on 15 ancestry-informative markers for parental populations and the population studied.

dbSNP ID	Chromosome	Allele	AFR*	AMR*	EUR*	OP	White	Light-brown	Dark-brown	Black
rs2065160	1	C	0.487	0.875	0.088	0.248	0.208	0.224	0.300	0.500
rs1240709	1	A	0.050	0.103	0.766	0.417	0.543	0.448	0.250	0.000
rs2814778	1	G	0.983	0.018	0.006	0.433	0.239	0.434	0.663	0.750
rs3796384	3	C	0.783	0.875	0.154	0.448	0.250	0.431	0.613	0.500
rs2278354	5	T	0.703	0.839	0.120	0.644	0.864	0.651	0.513	0.625
rs267071	5	C	0.088	1.000	0.654	0.412	0.457	0.401	0.350	0.125
rs1480642	6	C	0.121	0.621	0.993	0.658	0.717	0.696	0.538	0.500
rs222541	6	G	0.971	0.018	0.257	0.607	0.608	0.578	0.663	0.750
rs803733	9	C	0.013	0.410	0.880	0.522	0.739	0.519	0.413	0.500
rs7349	10	T	0.969	0.000	0.067	0.314	0.152	0.299	0.425	0.875
rs730570	14	A	0.197	0.054	0.896	0.542	0.688	0.528	0.525	0.250
rs1129038	15	C	0.996	0.983	0.224	0.810	0.409	0.809	0.900	1.000
rs1426654	15	C	0.967	0.931	0.013	0.409	0.087	0.356	0.738	0.875
rs730086	17	C	0.063	1.000	0.667	0.452	0.478	0.514	0.275	0.250
rs6034866	20	A	0.954	0.143	0.083	0.527	0.500	0.505	0.625	1.000

AFR = African; AMR = Amerindian; EUR = European; OP = Ouro Preto. *African, Amerindian, and European frequencies were described by Lins et al., 2010.

The admixture proportions (estimated with the AIMs) of the total population, regardless of skin color and after categorizing according to self-reported skin color, are shown in Table 2. In addition to the four reported skin colors originally provided in the questionnaire (white, light brown, dark brown, and black), volunteers were also categorized into two further skin color groups (light brown + dark brown and dark brown + black) owing to possible uncertainties about skin tone. The population of Ouro Preto showed a predominant contribution of

European ancestry (EUR = 0.503-0.539), followed by African (AFR = 0.333-0.425) and Amerindian (AMR = 0.036-0.164) ancestries according to the STRUCTURE and ADMIXMAP programs (Table 2). The results obtained with ADMIXMAP correlated with the estimates obtained with STRUCTURE (Spearman correlation: AFR $\rho = 0.90$, $P < 0.001$; EYP $\rho = 0.91$, $P < 0.001$; AMP $\rho = 0.51$, $P < 0.0017$). In addition, white and light-brown groups were predominantly of European ancestry (EUR = 0.675-0.704 and EUR = 0.521-0.555, respectively), whereas the black skin group was predominantly of African ancestry (AFR = 0.671-0.689; Table 2). However, when individuals with light-brown/dark-brown skin or dark-brown/black skin were grouped, no predominant ancestry contribution was found.

Table 2. Population admixture estimation for the whole population and for the self-reported skin color groups.

Self-reported skin color	Ancestral roots by STRUCTURE program			Ancestral roots by ADMIXMAP program		
	AFR	AMR	EUR	AFR	AMR	EUR
		Means ± SD Standard error (95%CI)			Means ± SD Standard error (95%CI)	
All (N = 189)	0.333 ± 0.203 0.015 (0.304-0.362)	0.164 ± 0.135 0.010 (0.145-0.183)	0.503 ± 0.230 0.017 (0.470-0.536)	0.425 ± 0.152 0.011 (0.403-0.447)	0.036 ± 0.020 0.001 (0.033-0.039)	0.539 ± 0.153 0.011 (0.517-0,561)
White (N = 24)	0.180 ± 0.129 0.026 (0.128-0.232)	0.116 ± 0.104 0.021 (0.074-0.158)	0.704 ± 0.187 0.038 (0.629-0.779)	0.298 ± 0.113 0.023 (0.207-0.389)	0.026 ± 0.009 0.002 0.022-0.030)	0.675 ± 0.115 0.023 (0.629-0.721)
Light-brown (N = 110)	0.314 ± 0.184 0.018 (0.280-0.348)	0.164 ± 0.136 0.013 (0.139-0.189)	0.521 ± 0.192 0.018 (0.485-0.557)	0.406 ± 0.130 0.012 (0.382-0.430)	0.038 ± 0.023 0.002 (0.034-0.042)	0.555 ± 0.130 0.012 (0.531-0.579)
Dark-brown (N = 40)	0.476 ± 0.184 0.029 (0.419-0.533)	0.188 ± 0.150 0.024 (0.142-0.234)	0.336 ± 0.221 0.035 (0.268-0.404)	0.551 ± 0.131 0.021 (0.510-0.592)	0.038 ± 0.017 0.003 (0.033-0.043)	0.412 ± 0.129 0.020 (0.372-0.452)
Black (N = 4)	0.671 ± 0.108 0.054 (0.565-0.777)	0.164 ± 0.064 0.032 (0.101-0.227)	0.166 ± 0.098 0.049 (0.070-0.262)	0.689 ± 0.048 0.024 (0.642-0.736)	0.026 ± 0.007 0.004 (0.019-0.033)	0.285 ± 0.053 0.026 (0.233-0.337)
Light-brown + dark-brown (N = 150)	0.357 ± 0.197 0.016 (0.325-0.389)	0.171± 0.140 0.011 (0.149-0.193)	0.472 ± 0.216 0.018 (0.437-0.507	0.445 ± 0.144 0.012 (0.422-0.468)	0.038 ± 0.021 0.002 (0.035-0.041)	0.517 ± 0.144 0.012 (0.494-0.540)
Dark-brown + black (N = 44)	0.493 ± 0.187 0.028 (0.438-0.548)	0.186 ± 0.144 0.017 (0.143-0.229)	0.321 ± 0.218 0.033 (0.257-0.385)	0.563 ± 0.132 0.020 (0.524-0.602)	0.036 ± 0.017 0.003 (0.031-0.041)	0.400 ± 0.129 0.020 (0.362-0.438)

AFR = African; AMR = Amerindian; EUR = European; SD = standard deviation; CI = confidence interval.

The mean contribution of African ($F \geq 15.5$) and European ($F \geq 17.6$) ancestries differed significantly among all self-reported skin color groups (ANOVA, $P < 0.001$), but there were no significant differences in Amerindian ancestry ($F \geq 1.4$; $P \geq 0.123$) among the groups. However, the Games-Howell post-hoc test (Table 3) showed that the contribution of African ($P = 0.086$) and European ($P = 0.098$) ancestries did not differ between black and dark-brown groups. Similarly, the contribution of Amerindian ancestry did not differ significantly among the skin-color groups tested.

The box plot of admixture estimates (mean ± standard deviation) for the white, light-brown, and dark-brown + black skin color groups in the Ouro Preto population is shown in

Figure 1. This analysis was performed for only the former three groups because there were significant differences in the contribution of African and European ancestry among all skin color groups, and because the observed proportions for the light-brown (61.8%) and dark-brown + black (24.7%) groups were closer to those described by IBGE for the Ouro Preto population in 2010 (52.5% and 14.4%, respectively). In fact, the contribution of each ancestry overlapped in all skin color groups, except for African ancestry in the white and dark-brown + black groups (Figure 1).

Table 3. Ancestry estimation comparison between self-reported skin color groups using STRUCTURE program.

Dependent variable	Group (i)	Group (j)	Mean difference (i - j)	Standard error	P value	95% Confidence interval	
						Lower bound	Upper bound
AFR	White	Light brown	-0.134	0.032	0.001	-0.219	-0.050
		Dark brown	-0.296	0.039	<0.001	-0.399	-0.192
		Black	-0.491	0.060	0.003	-0.721	-0.260
		Light-brown + dark-brown	-0.177	0.031	<0.001	-0.252	-0.102
		Dark-brown + black	-0.313	0.039	<0.001	-0.406	-0.221
	Black	Light brown	0.356	0.057	0.015	0.113	0.599
		Dark brown	0.195	0.061	0.086	-0.033	0.423
		Light-brown + dark-brown	0.313	0.056	0.016	0.099	0.527
	Light Brown	Dark brown	-0.161	0.034	<0.001	-0.251	-0.072
AMR	White	Light brown	-0.048	0.025	0.227	-0.115	0.018
		Dark brown	-0.072	0.032	0.118	-0.156	0.012
		Black	-0.048	0.038	0.619	-0.179	0.084
		Light-brown + dark-brown	-0.055	0.024	0.073	-0.113	0.004
		Dark-brown + black	-0.070	0.030	0.063	-0.143	0.003
	Black	Light brown	-0.000	0.034	1.000	-0.139	0.138
		Dark brown	-0.024	0.040	0.925	-0.155	0.106
		Light-brown + dark-brown	-0.007	0.034	0.978	-0.130	0.116
	Light Brown	Dark brown	-0.024	0.027	0.814	-0.095	0.048
EUR	White	Light brown	0.183	0.042	0.001	0.069	0.297
		Dark brown	0.368	0.052	<0.001	0.231	0.505
		Black	0.539	0.062	<0.001	0.335	0.742
		Light-brown + dark-brown	0.232	0.042	<0.001	0.129	0.335
		Dark-brown + black	0.383	0.050	<0.001	0.262	0.505
	Black	Light brown	-0.356	0.053	0.009	-0.573	-0.139
		Dark brown	-0.171	0.060	0.098	-0.373	0.032
		Light-brown + dark-brown	-0.306	0.052	0.011	-0.497	-0.116
	Light Brown	Dark brown	0.185	0.039	<0.001	0.081	0.289

AFR = African; AMR = Amerindian; EUR = European.

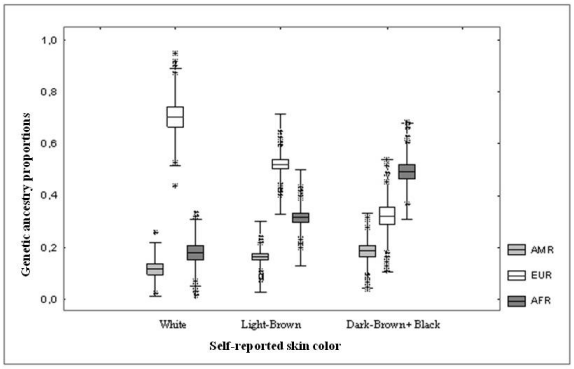

Figure 1. Box plot of the population admixture estimation among the self-reported skin color groups: white, light-brown, and dark-brown + black. AFR = African, AMR = Amerindian, EUR = European. *outliers.

DISCUSSION

The genetic ancestry proportions for the Ouro Preto population (EUR = 0.503-0.539, AFR = 0.333-0.425, and AMR = 0.036-0.164) are consistent with the history of colonization in the region. In fact, a predominant European influence was previously reported in the state of Minas Gerais in cities such as Montes Claros (0.52) and Manhuaçu (0.73) (Silva et al., 2010), as well as in southeastern Brazil (0.56-0.799) (Callegari-Jacques et al., 2003; Lins et al., 2010, Pena et al., 2011; Giolo et al., 2012) and in the entire country (0.707-0.77) (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011), indicating a recent and substantial European immigration. Moreover, the African contribution (0.333-0.425) in Ouro Preto was found to be higher than that in southeast Brazil (0.141-0.27) (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011; Giolo et al., 2012). The African contribution in Montes Claros (0.39) (Silva et al., 2010) is also higher than that in southeast Brazil (0.141-0.27) (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011; Giolo et al., 2012), which may reflect the large flow of individuals of African origin to the state of Bahia where the African proportional contribution was reported at 0.29 (Pena et al., 2011). Similarly, the Federal District is another Brazilian locality with a relatively high contribution of African ancestry (0.21-0.254) compared to the overall contribution estimated for its geographically correlated region (the African ancestry contribution in Midwestern Brazil is approximately 0.18) (Callegari-Jacques et al., 2003; Lins et al., 2010; Lins et al., 2011; Leite et al., 2011). These data indicate that if the mean African ancestry contribution found for southeastern Brazil were extrapolated to Ouro Preto and Montes Claros, or if data from Midwest Brazil were extrapolated to the Federal District, the African contribution would be underestimated in these cities. The same phenomenon may occur in other Brazilian cities as most studies have determined ancestry proportions in Brazilian regions using samples of individuals from the capital and/or from large urban centers (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011).

We found significant differences in the mean values of African and European ancestry when comparing the ancestry contribution among self-reported skin color groups. In fact, on average, self-reported white individuals had a higher European contribution, whereas self-reported black individuals had lower values. In addition, an opposite result was found with respect to African contribution, whereas the other self-reported skin color categories showed intermediate European and African contribution values. However, we observed considerable dispersion within each group and substantial overlap in ancestry between groups when the standard deviation was considered (Figure 1). Similar results were found in other studies investigating sample populations from Brazil (Parra et al., 2003; Pimenta et al., 2006; Suarez-Kurtz et al., 2007; Santos et al., 2009; Lins et al., 2011; Pena et al., 2011).

The small number of AIMs used to infer ancestry proportions is a limitation of our study. However, previous studies in sample populations from Brazil using 12-40 AIMs found small variations in the proportions of European, African, and Amerindian ancestry, and these variations were consistent at the population level (Callegari-Jacques et al., 2003; Lins et al., 2010; Pena et al., 2011). Thus, we believe that the 15 AIMs used in our study are informative markers that can effectively estimate population admixture. Two other studies on the Federal District population (Midwest region) using 13 and 21 AIMs respectively also showed small variations in ancestry proportions (Lins et al., 2011; Leite et al., 2011).

In conclusion, we demonstrated that the ancestry proportions of the Ouro Preto popu-

lation are different from those of southeast Brazil, which emphasizes the need to study other Brazilian cities. Moreover, we showed that self-reported skin color classifications may be appropriate for describing the population structure of Ouro Preto as far as European and African genetic ancestries are concerned.

Conflicts of interest

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

Research supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, #47.4965/2004-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; EDT-#325/05, EDT-CDS #770/05, CDS-APQ-#00519-09, CBB-APQ-#02260-10), Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF), and Pró-Reitoria de Pós-Graduação e Pesquisa from Universidade Federal de Ouro Preto and from Universidade Católica de Brasília (PRPGP-UCB), Brazil. We thank Julio Cesar Rodrigues Fontenelle for suggestions and statistical analysis.

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